Please delete the paragraph on page 16, lines 24-25, and replace it with the following paragraph:

a3

FIGURE 8 (SEQ ID NOS 3-5, respectively, in order of appearance) shows a scheme for vector preparation for ligase independent cloning method using site specific nickases.

Please delete the paragraph on page 16, lines 27-28, and replace it with the following paragraph:

a4

FIGURE 9 (SEQ ID NOS 6-8, respectively, in order of appearance) shows a scheme of preparation of covalently closed DNA molecules using site specific DNA nickases.

Please delete the paragraph on page 17, lines 15-25, and replace it with the following paragraph:

a5

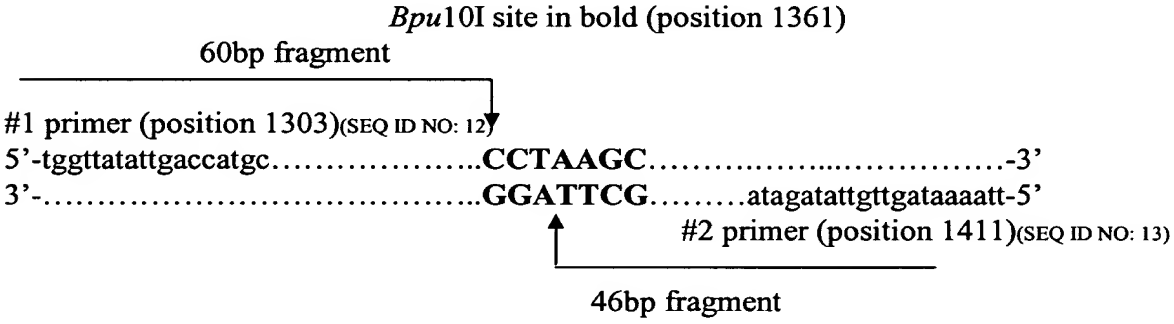
In order to confirm this proposition point mutations inactivating catalytic activity but retaining protein-protein interaction were introduced into α and β subunits of *Bpu*10I Enase. For this purpose amino acid sequences of both subunits were analysed trying to identify catalytic centres analogous to those found in other RE, e.g. motif (E/D)$X_{9\text{-}15}$EXK (SEQ ID NO: 9) characteristic for some RE, and the $SD^{171}X_8E^{180}XK^{182}$ (SEQ ID NO: 10) motif in the N-terminal domain of *Bpu*10I α and $TE^{168}X_8E^{177}XK^{179}$ (SEQ ID NO: 11) motif in the N-terminus of β subunit that corresponded quite well to the consensus one were identified. On the basis of these observations the presumption may be made that (E/D)X_8EXK (SEQ ID NO: 18) motifs might be the catalytic/magnesium binding centres of the proteins described therein. This region was chosen as the target for point mutagenesis and series of mutations were introduced by standard PCR techniques both into α and β subunits (Fig. 1, in which the positions where amino acid substitutions were introduced are underlined.).

Please delete the paragraph on page 18, line 8 to page 19, line 16 and replace it with the following paragraph:

a6

E180Q mutant of α subunit and E177A mutant of β subunit were chosen for further experiments. Proteins were purified to near homogeneity (see Example 1) and it was shown by several different methods (see Example 2, 3) that when E180Q mutant of α subunit

(αE180Q) and native β subunit are combined in the reaction mixture the only one strand with the sequence 5'-CC^TNAGC-3' is effectively nicked. *Vice versa*, the presence of native α subunit and E177A mutant of β subunit (βE177A) in the reaction mixture results in the specific nicking of the opposite DNA strand with the recognition sequence 5'-GC^TNAGG-3' (Fig. 3). The experiment was performed as follows: ΦX174 plasmid DNA, a set of specific primers (#1:5'TGGTTATATTGACCATGC3' (SEQ ID NO: 12), position 1303; #2:5'TTAAAATAGTTGTTATAGATA3' (SEQ ID NO: 13), position 1411), dNTPs and α[^{35}dATP] were used in the extension reaction with T7 DNA polymerase through unique *Bpu*10I recognition site (position 1361). After inactivation of polymerase by heating at 65°C for 15 min. labelled extension products were digested in parallel with Bpu10I restriction endonuclease, N.*Bpu*10Iα(α+βE177A) and N.*Bpu*10Iβ(αE180Q+β). Digestion reactions were analysed by 10% denaturing PAGE. Due to different distance from the *Bpu*10I recognition site to the primer annealing sites (60 bp and 46 bp) on the top and bottom DNA strands the cleaved strand could be specifically identified on the gel. In Figure 3, lane 1 shows labelled ΦX174 DNA digested with *Bpu*10I restriction endonuclease, lane 2 shows labelled ΦX174 DNA digested with N.*Bpu*10Iα (α+βE177A), and lane 3 shows labelled ΦX174 DNA digested with N.*Bpu*10Iβ (αE180Q+β). Primers and fragments cleaved with each nickase are shown below:



Thereby obtained data confirm the hypothesis that a restriction endonuclease recognizing a non-palindromic DNA sequence can be converted by site - specific (or non specific) mutagenesis into a specific nickase cleaving only one DNA strand. These data also confirm the presumption that in the native R.*Bpu*10I enzyme, the α subunit is responsible for cleavage of DNA strand with the sequence 5'-GC^TNAGG-3', while the β subunit - with the sequence 5'-CC^TNAGC-3'.

Please delete the paragraph on page 27, line 14 to page 28, line 8 and replace it with the following paragraph:

Example 4

Vector preparation for ligation independent cloning method.

pUC57-PKA2 vector plasmid possessing *Pst*I recognition site flanked by two inverted *Bpu*10I recognition sites was constructed by ligating the following cassette into *Pae*I-*Kpn*I digested pUC57 (Fermentas):

```
                                PstI                  Bpu10Iα
    5'-CCTAAGCTCACTCTCAATGGTCTGCAGAGGTCAGACACGCTTAGGCATG-3'(SEQ ID NO: 14)
3'-CATGGGATTCGAGTGAGAGTTACCAGACGTCTCCAGTCTGTGCGAATCC-5' (SEQ ID NO: 15)
      Bpu10Iα
```

20 µg of plasmid pUC57-PKA2 were treated with N.*Bpu*10Iα (α+βE177A) cleaving 5'-GC^TNAGG-3' prepared as described in Example 1 in Bpu10I buffer in 400 µl total reaction volume at 37°C for 3 hours. Nicking enzyme concentration in the reaction mixture was 2pmol/µl. After nicking reaction was complete as confirmed by agarose gel electrophoresis 10 units of *Pst*I restriction endonuclease were added into reaction mixture and incubation was prolonged at 37°C for another 2 hours. The reaction was terminated by phenol/chloroform deproteinisation followed by DNA precipitation with ethanol. Digested vector DNA was then incubated with 500 u of *E.coli* Exonuclease III in Exo III buffer (Fermentas) at 25°C for 1 min. Exonuclease degradation should result in the formation of the vector molecule possessing 18 nt and 21 nt single stranded protruding 3' ends.

Please delete the paragraph on page 28, lines 10-14 and replace it with the following paragraph:

Following uridinilated primers possessing 5' ends complementary to vector DNA sequence and their 3' ends complementary to phage lambda sequence (EMBL/GenBank Acc. No. J02459, 2761-3678) have been synthesized:

Seq #1: 5'-CGUGUCUGACCUGAAAAAATA-3' (SEQ ID NO: 16)

Seq #2: 5'-GCUCACUCUCAAUGGTGGCGG-3' (SEQ ID NO: 17)